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A Highly Selective OFF-ON Red-Emitting Phosphorescent Thiol Probe with Large Stokes Shift and Long Luminescent Lifetime

Shaomin Ji,[†] Huimin Guo,[†] Xiaolin Yuan,[‡] Xiaohuan Li,[‡] Haidong Ding,[‡,§] Peng Gao,[⊥] Chunxia Zhao,[⊥] Wenting Wu,[†] Wanhua Wu,[†] and Jianzhang Zhao*[,†]

State Key Laboratory of Fine Chemicals, School of Chemical Engineering, 158 Zhongshan Road, Dalian University of Technology, Dalian 116012, P. R. China, Center Laboratory, Affiliated Zhongshan Hospital of Dalian University, Dalian 116001, P. R. China, Department of Immunology, Harbin Medical University, 194 Xuefu Road, Harbin 150081, P. R. China, and National Chromatographic R & A Center, Dalian Institute of Chemical Physics, Chinese Academy of Science, 457 Zhongshan Road, Dalian 116023, P. R. China.

zhaojzh@dlut.edu.cn

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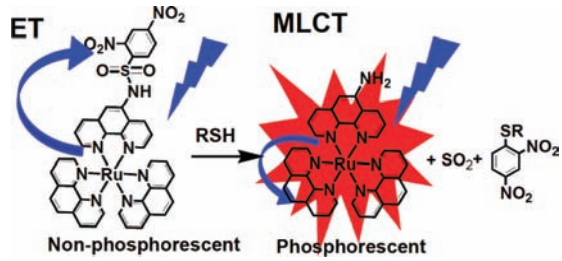
ABSTRACT



An OFF-ON red-emitting phosphorescent thiol probe is designed by using the ^{3}MLCT photophysics of Ru(II) complexes, i.e., with Ru(II) as the electron donor. The probe is non-luminescent because the MLCT is corrupted by electron transfer from Ru(II) to an intramolecular electron sink (2,4-dinitrobenzenesulfonyl). Thiols cleave the electron sink, and the MLCT is re-established. Phosphorescence at 598 nm was enhanced by 90-fold, with a 143 nm (5256 cm^{-1}) Stokes shift and a 1.1 μs luminescent lifetime.

Thiols, such as glutathione and cysteine, play pivotal roles in living organisms, and thus fluorescent molecular probes for selective detection of thiols are of great interest.[1−7] Various mechanisms have been used for design of fluorescent thiol probes, e.g., attaching electrophilic groups to fluorophores (iodoacetamides or benzyl halides),[8] fluorophores with maleimide appendents, etc.[4,9−11] More recently, fluorophores containing a -CHO group were also used.[5,12−16] As a new sensing motif, fluorophores protected by 2,4-dinitrobenzenesulfonyl (DNBS) have been used for thiol probes, which

[†] Dalian University of Technology.
[‡] Affiliated Zhongshan Hospital of Dalian University.
[§] Harbin Medical University.
[⊥] Dalian Institute of Chemical Physics.

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demonstrated perfect emission OFF-ON switch effect and good selectivity.[1,2,17,18] With DFT calculations we demonstrated that DNBS induces a dark excited state to the probe (S_1, the transition oscillator strength for $S_0 \rightarrow S_1$ is close to zero; thus $S_0 \rightarrow S_1$ is a forbidden transition and $S_1 \rightarrow S_0$ relaxation is nonradiative),[17] and thus the probe is nonfluorescent.[17,19] Cleavage of the electron sink DNBS with thiols will re-establish the radiative S_1 state of the fluorophore (oscillator strength of $S_0 \rightarrow S_1$ is close to unity, indicating the transition is allowed and $S_1 \rightarrow S_0$ is probably a radiative transition), and thus the emission is switched on.[17]

However, most of the probes are based on fluorescence,[1] for which the singlet excited state is the emissive state (usually S_1, Kasha's rule).[11,17] These thiol probes show some drawbacks: (1) small Stokes shift, e.g., probes based on BODIPY or rhodamines;[4,5] (2) short excitation−emission wavelength, which induces undesired background fluorescence;[20] (3) microenvironment-sensitive emission, e.g., being effected by pH or polarity of the media;[16] and (4) short luminescent lifetimes (ns, 10^{-9} s, due to the spin manifold of the $S_0 \rightarrow S_1$ transition),[11] and so it is difficult for these probes to be used for lifetime-based analysis.[11] Therefore, new thiol probes with drastically different lumophores are desired to tackle these problems.

Much investigation has been focused on binding sites and the luminophores of molecular probes. However, fewer efforts have been made to explore new sensing mechanisms.[16,21−23] Recently we have been interested in the study of molecular probes with new binding sites, chromophores, and new sensing mechanisms.[17,24−26]

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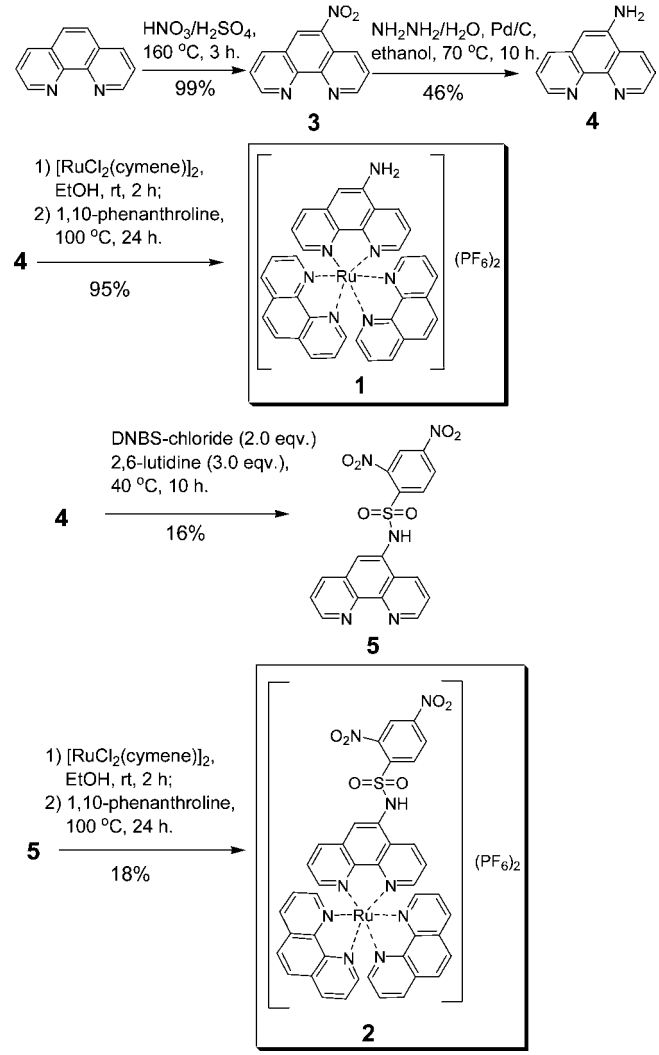
Previously we designed OFF-ON fluorescent thiol probes with DNBS as intramolecular electron sink.[17] However, our probes suffer from drawbacks: (1) the pyrene moiety is not an ideal electron donor; the nonefficient electron transfer (ET) deteriorates the quenching efficiency or the contrast ratio,[23b] and as a result, only 20- to 53-fold emission enhancement was observed;[17] (2) low emission quantum yield in aqueous solutions, which is common for most of the fluorophores;[11,17] and (3) short luminescent lifetimes (ns).[17]

To tackle the above limitations, herein we designed a thiol probe by using Ru(II) poly(1,10-phenanthroline) complex as the luminophore, which is known to show environment-nonsensitive metal-to-ligand charge transfer (^{3}MLCT) red emission (at ca. 600 nm), large Stokes shift (ca. 150 nm, 5250 cm^{-1}), and long luminescent lifetimes (μs, 10^{-6} s).[27,28] Thus we envisaged the new phosphorescent thiol probe **2** (Scheme 1).

Scheme 1. Syntheses of Complex **1** and Probe **2**



Probe **2** is based on tuning the MLCT photophysics by DNBS. The typical MLCT photophysics of the Ru(II) complex, i.e., the photoinduced ET from the Ru(II) center

(a *strong* electron donor) to the *N^N* coordination ligand, was perturbed by DNBS, a *strong* intramolecular electron acceptor. The ET destination will be diverted from the *N^N* ligand to DNBS, and thus MLCT will be corrupted and the emission will be switched off. Cleavage of the electron sink (DNBS) by thiols will re-establish the MLCT of the Ru(II) complex, with the *N^N* ligand as electron acceptor, and thus the ^{3}MLCT phosphorescence will be switched on.

The absorption bands of the complexes below 300 nm are dominated by the ligand-centered absorption ($^1\pi-\pi^*$, $S_0 \rightarrow {}^1$IL, intraligand singlet excited state) (Figure 1a). The

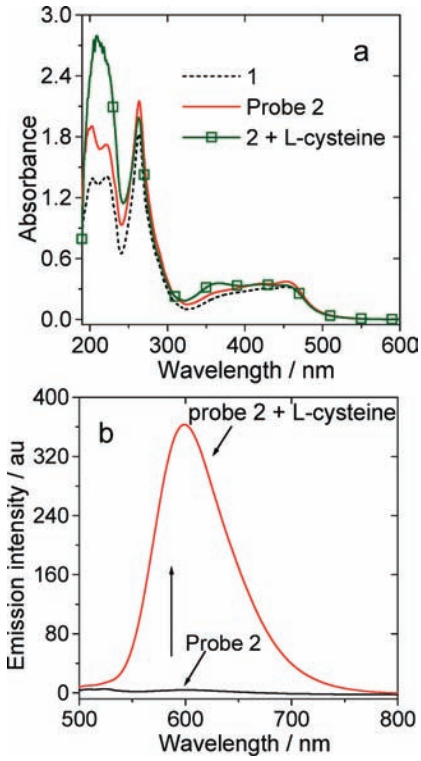


Figure 1. (a) UV−vis absorption of the complex **1** and probe **2** before and after addition of L-cysteine. (b) Phosphorescence emission spectra (λ_{ex} = 455 nm) of probe **2** before and after addition of L-cysteine. In acetonitrile/water (4:1 v/v) solution. The concentrations of the probe and L-cysteine are 2.0×10^{-5} mol dm^{-3} and 1.0×10^{-2} mol dm^{-3}, respectively. Temperature was 30 °C.

absorption in the visible range of the spectra at 400−500 nm are the MLCT bands ($S_0 \rightarrow {}^1$MLCT).[11,17,27,28]

Probe **2** is non-luminescent (Figure 1b) because of the corrupted MLCT by the efficient photoinduced ET between the potent electron donor (Ru center) and the strong electron acceptor (DNBS). Phosphorescence (598 nm) was switched on by cleavage of the DNBS with thiols; the enhancement is as high as 90-fold (Figure 1b) versus the 20- to 53-fold enhancement of our previous thiol probes with pyrene as electron donor.[17] The sensing mechanism was verified by mass spectrometry (see Supporting Information).[1,17] We attribute the improved emission enhancement in the presence of thiol to the greatly reduced background emission by the efficient ET, as well as the high luminescence quantum yield of the Ru(II) complex phosphore in aqueous solutions.[11,27,28]

To the best of our knowledge, probe **2** is the first phosphorescent probe with OFF-ON switch effect for selective thiol detection. A thiol-reactive Ru(II) complex with a maleimide group was reported,[3] but with significant background luminescence. An elegant *N∧N* platinum(II) bis(acetylide) complex containing -CHO groups was reported as cysteine probe, but the emission was decreased in presence of thiols and only minor red-shifting was observed.[7b] Moreover, the background emission of a trinuclear heterobimetallic Ru(II)/Pt(II) complex thiol probe is not negligible, and the emission enhancement is only ca. 15-fold in the presence of thiols.[29] Although a thiol probe based on squaraine dye with emission at 600 nm was reported, the emission enhancement is only ca. 6-fold.[30] In our case, probe **2** shows emission in the deep red range, and the emission enhancement is up to 90-fold. Furthermore, **2** shows a significant phosphorescence response only in the presence of thiols, thus good selectivity is observed (Figure 2).

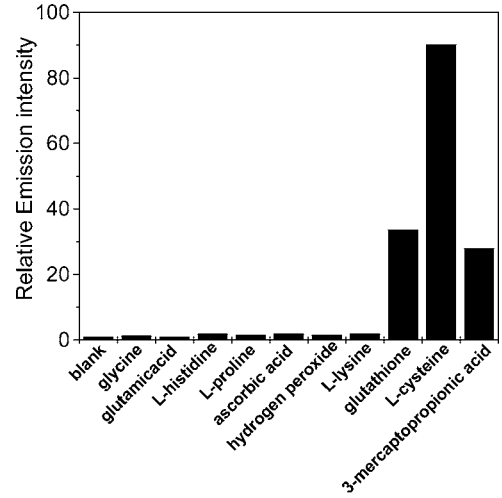


Figure 2. Response of probe **2** to different analytes. Relative fluorescence intensity of 20 μM probe at 598 nm (λ_{ex} =455 nm) before and after incubation in the presence of 10 mM analytes at 30 °C, pH 7.4, acetonitrile/water (4:1 v/v) solution.

The excited probe **2** shows biexponential decay kinetics, includes a fast decay process (0.5 ns) and is followed by a slow decay process (ca. 1.1 μs) (see Supporting Information).

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We propose the electron transfer from Ru(II) to DNBS in **2** as a two-step cascade process, i.e., the electron first transfers from Ru(II) to the *N*^*N* ligand and finally to DNBS.[2,17,18] The fast kinetics is probably due to the *N*^*N* ligand → DNBS ET process, and the long-lived decay is due to the ^{3}MLCT excited state. In the presence of thiols, the cleaved product, i.e., **1**, shows intensive phosphoresence at 598 nm, with a luminescent lifetime of 1.1 μs (Supporting Information).

We have been interested in studying the sensing mechanism of molecular probes with DFT calculations.[17,24−26] The HOMO and LUMO orbitals of **2** were calculated (Figure 3).[31] ET from Ru(II) center to DNBS was observed, which

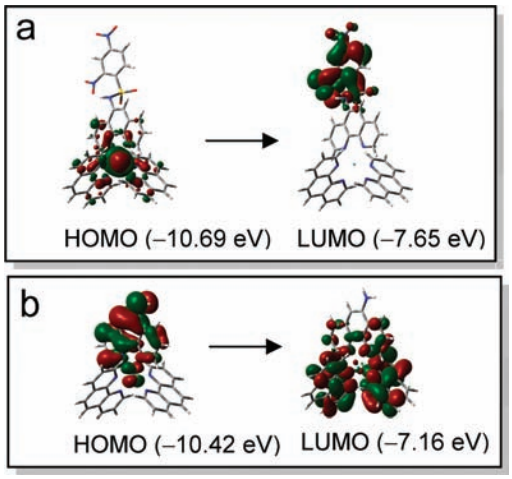

Figure 3. Frontier molecular orbitals (MOs) of probe **2** before and after cleavage by thiols. (a) HOMO and LUMO of probe **2**. (b) HOMO and LUMO of **1** (i.e., the cleavage product of **2** by thiols). The energy levels of the MOs are shown. Calculation is based on the ground state geometry by DFT at the B3LYP/6-31G(d)/LanL2DZ level using Gaussian 09.[32]

leads to quenching of the phosphorescence of probe **2**.[17,19] Conversely, ligand-to-ligand charge transfer (LLCT) and MLCT were found for **1**, i.e., the cleavage product of **2** by thiols (Figure 3b). The electronic transition of **1** is consistent with the typical photophysics of the Ru(II) polypyridine complexes.[11,27,28] Thus, the phosphorescence of probe **2** will be switched ON in the presence of thiols.

Imaging of intracellular thiols of NCI-H446 cells with probe **2** was carried out (Figure 4). The cells were incubated with **2**, and red emission was observed with 488 nm laser excitation (Figure 4d). In order to prove that probe **2** is specific to the intracellular thiols, we used *N*-methylmaleimide to pretreat cells to remove the intracellular thiols,[17] then the cells were incubated with probe **2**, but no red phosphorescence was observed (Figure 4g and i). These results confirm that **2** is specific for thiols over other analytes in living cells.

In conclusion, a highly selective OFF-ON red-emitting phosphorescent thiol probe has been designed by using the

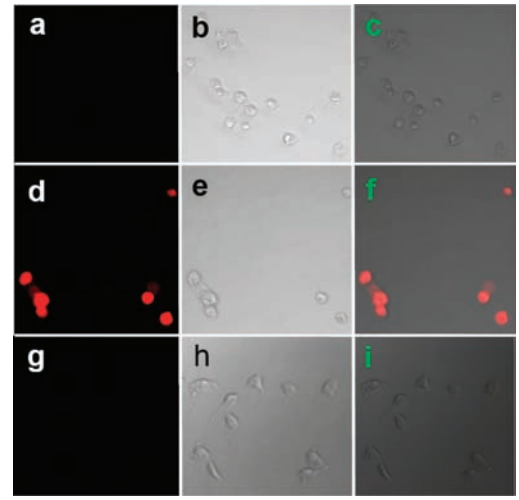

Figure 4. Luminescence images of NCI-H446 cells. (a) Images of cell. (d) Images of cells incubated with probe **2** (30 μM) for 8 h at 37 °C. (g) Images of cells pretreated with *N*-methylmaleimide (0.5 mM) for 1 h at 37 °C and then incubated with probe **2** (30 μM) for 8 h at 37 °C. (b, e, h) Bright field images corresponding to images a, d, g. (c) Overlay of images a and b. (f) Overlay of images d and e. (i) Overlay of images g and h.

strong intramolecular electron acceptor DNBS to modulate the intrinsic photophysics of the Ru(II) poly(1,10-phenanthroline) complex, i.e., the metal-to-ligand charge transfer (MLCT) with Ru(II) as the electron donor. The MLCT of the probe is corrupted by the ET from Ru to DNBS, and thus the phosphorescence is quenched. Thiols cleave the DNBS, the MLCT process is re-established, and thus the phosphorescence is switched on, with emission enhancement up to 90-fold. The emission of the thiol detection with probe **2** is featured with long emission wavelength (598 nm), large Stokes shift (143 nm, 5256 cm^{-1}), and long luminescent lifetime (1.1 μs). Luminescent imaging of intracellular thiols were performed. The design rational of the phosphorescent thiol probe **2** by tuning the MLCT of Ru(II) complexes with intramolecular electron sink will be helpful for development of OFF-ON *phosphorescent* probes, which usually show longer emission wavelength, larger Stokes shift, and much longer luminescent lifetimes compared to the *fluorescent* molecular probes.

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Supporting Information Available: General experimental methods, ^{1}H and ^{13}C NMR data, and spectra of the complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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